UNCLASSIFIED
SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

1a. REPORT SECURITY CLASSIFICATION: Unclassified	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY: —	3. DISTRIBUTION/AVAILABILITY OF REPORT: Approved for public release; distribution is unlimited
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S): NMRI 90-55	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION: Naval Medical Research; 6b. OFFICE SYMBOL (If applicable)	7a. NAME OF MONITORING ORGANIZATION: Naval Medical Command
6c. ADDRESS (City, State, and ZIP Code): Bethesda, Maryland 20814-5055	7b. ADDRESS (City, State, and ZIP Code): Department of the Navy, Washington, D.C. 20372-5120
8a. NAME OF FUNDING/SPONSORING ORGANIZATION: Naval Medical Research and Development Command; 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER
8c. ADDRESS (City, State, and ZIP Code): Bethesda, Maryland 20814-5055	10. SOURCE OF FUNDING NUMBERS: PROGRAM ELEMENT NO. N.A.; PROJECT NO.; TASK NO.; WORK UNIT ACCESSION NO

11. TITLE (Include Security Classification)
Similarity in Pathogenic Features in Lung and Peritoneal Infection by Coxiella burnetii, Typhus Group Rickettsiae, and Chlamydiae.

12. PERSONAL AUTHOR(S)
Khavkin T., Weiss E.

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
journal article	FROM ____ TO ____	1990	19

16. SUPPLEMENTARY NOTATION
Reprinted from: Rickettsiology: urrent Issues and Perspectives, Vol.590 of the Annals of the New York Academy of Sciences, June 26, 1990, p. 85-103

17. COSATI CODES		
FIELD	GROUP	SUB-GROUP

18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
Proteobacteria; Coxiella burnetii; Typhus group rickettsiae; Chlamydia; Divergent evolution; Cell-pathogen interaction; Respiratory epithelium; Peritoneal mesothelium; Squamous epithelium

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT: [X] UNCLASSIFIED/UNLIMITED [ ] SAME AS RPT. [ ] DTIC USERS	21. ABSTRACT SECURITY CLASSIFICATION: Unclassified
22a. NAME OF RESPONSIBLE INDIVIDUAL: Phyllis Blum, Information Services Division	22b. TELEPHONE (Include Area Code): 202-295-2188; 22c. OFFICE SYMBOL: ISD/ADMIN/NMRI

Reprinted from Rickettsiology: Current Issues and Perspectives
Volume 590 of the *Annals of the New York Academy of Sciences*
June 26, 1990

Similarity in Pathogenic Features in Lung and Peritoneal Infection by *Coxiella burnetii*, Typhus Group Rickettsiae, and Chlamydiae[a]

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INTRODUCTION

The basic trend of evolution, divergent development of organisms, is known to be accompanied by the opposite trend: the appearance of secondary convergence of characteristics among phylogenetically unrelated species. In the world of pathogenic microorganisms, these trends are manifested by dissimilarity between closely related species and by similarity in interaction with the animal host by unrelated species. Both trends are especially apparent in microorganisms adapted to reside on or within specialized cells of the host, a highly specialized ecological habitat.[1,2] The existence of enteroinvasive, enteropathogenic, and toxigenic serotypes of the same species, *Escherichia coli*,[2] is an example of divergent evolution. On the other hand, the ability of unrelated species, such as the protozoan *Toxoplasma gondii*, the bacterium *Mycobacterium tuberculosis*, and *Chlamydia* to inhabit macrophage phagosomes while preventing phagosome-lysosome fusion[3] provides examples of convergent evolution of microorganisms with regard to basic determinants of pathogenicity. The analysis of both trends of the evolution of microorganisms is important for understanding the pathogenesis of the diseases they cause. This paper attempts to call attention to the remarkable similarity in cell-pathogen interaction among phylogenetically unrelated microorganisms, *Coxiella burnetii*, typhus group rickettsiae, and *Chlamydia*. The first two belong to the α and γ subdivisions, respectively, of the Proteobacteria,[4] and *Chlamydia* to an independent eubacterial group.[5] These pathogens also interact differently with their host cell: *C. burnetii* multiplies in the phagolysosomes, typhus group rickettsiae in the cytoplasm, and *Chlamydia* in the phagosome.[6] Despite these differences, all these pathogens are capable of gaining access into and residing within the same specialized cells, the respiratory epithelium and peritoneal meso-

[a]This review includes experiments performed by Theodor Khavkin and his colleagues at the Pasteur Institute, Leningrad, USSR, and published only in the Russian language, as well as some unpublished experiments with F. Krasnik. The preparation of this review has been supported by Interferon Sciences, Inc.

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thelium, which together with the vascular endothelium comprise a cell type known as the simple squamous epithelium.[7] Such a similarity determines some common features in the pathogenesis and clinical manifestations of the respective infections. The interaction of *Coxiella*, typhus group rickettsiae, and *Chlamydia* with cells of the simple squamous epithelia in experimental infectious processes is the primary subject of this review.

INFECTION OF THE RESPIRATORY EPITHELIUM

C. burnetii *Infection*

In humans, *C. burnetii* infection—Q fever—is not infrequently associated with so-called atypical pneumonia, which may emerge after the incubation period ranging from 10 to 20 days and sometimes presents as an incidental radiographic finding without obvious clinical manifestations.[8] Because of relatively low (50%) pulmonary involvement among human volunteers and in guinea pigs infected with *C. burnetii* by inhalation, Tiggertt *et al.*[9] have presumed that *C. burnetii* is not well adapted to reside in the cells of the respiratory tract and that the lung presents only a portal of entry of the pathogen into the body. More recent studies of intranasal challenge of mice and guinea pigs[10,11] have shown, however, that *C. burnetii* does inhabit cells of the respiratory parenchyma, pneumocytes as well as pulmonary fibroblasts, and histiocytes.

In guinea pigs and in mice, *C. burnetii* infection develops differently, as an overt disease and as an inapparent one, respectively. However, local infectious processes in these animals, in general, are, similar.[12] In the lungs, coxiellae reside initially within phagolysosomes of susceptible cells (FIG. 1A), without apparent cell damage and inflammatory response. The response, for the most part, is an influx of polymorphonuclear leukocytes (PMN), which develops in the area of destroyed infected cells. It is then transformed into a macrophage- and lymphocyte-dominated pneumonia, which subsides with complete resolution. Similar pulmonary lesions have been found in animals infected with *C. burnetii* via intraperitoneal or testicular challenge, with subsequent hematogenous involvement of the lungs.[12]

Limited damage to the vascular endothelium is observed in areas of acute inflammation (FIG. 1B). The damage is not associated with invasion of coxiellae into the endothelium. It is considered a consequence of a local effect of lipopolysaccharide (LPS) released from degraded microorganisms in the pneumonic exudate.[10,11]

Infection with Typhus Group Rickettsiae

Pulmonary involvement in environmentally acquired human epidemic and endemic typhus is relatively infrequent, in contrast to its occurrence in some laboratory models for the disease. It is not clear, however, whether pneumonia in typhus represents only a secondary bacterial complication or involves the etiologic agents of the disease, *Rickettsia prowazekii* and *R. typhi*. Davydovsky[13] and, later, Allen and Spitz[14] assumed that the early pulmonary lesions in epidemic typus had a rickettsial etiology, since the histologic pattern of these lesions, for

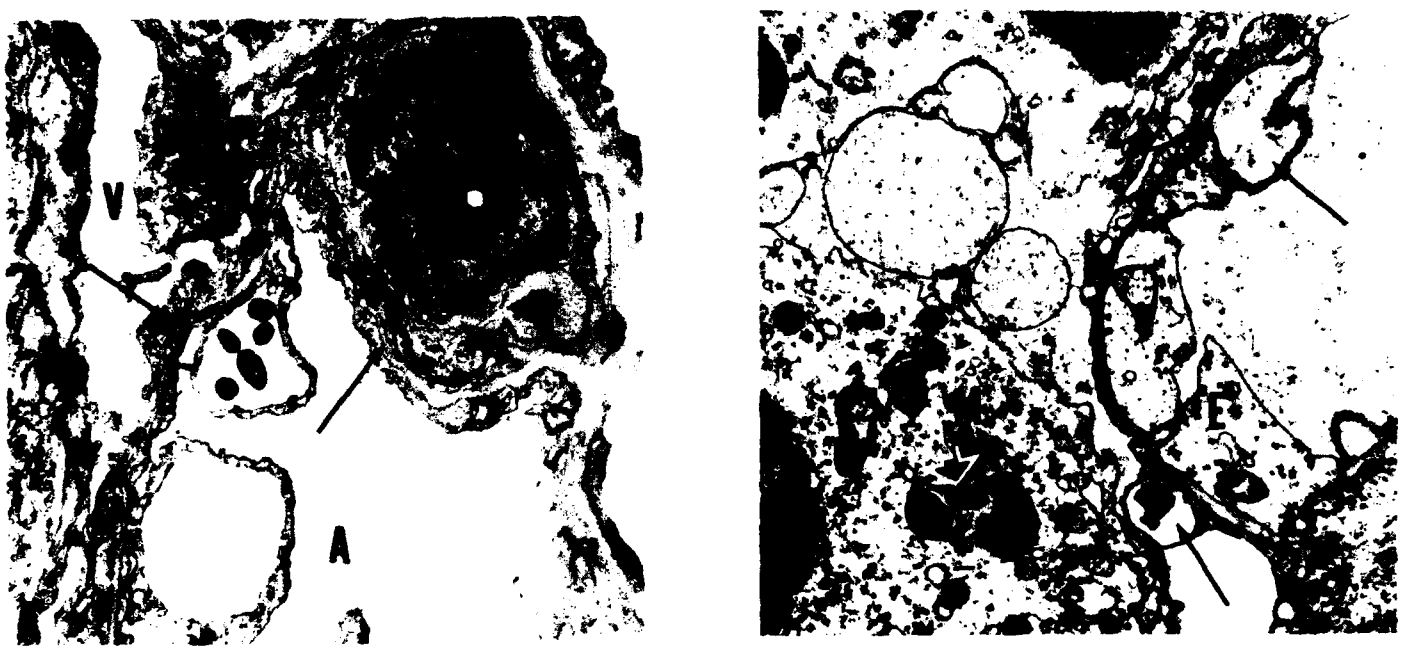

FIGURE 1. Cells containing *C. burnetii* (*Apodemus microtii*, phase I strain) and signs of indirect damage to the respiratory parenchyma in mouse lungs. **(A)** Type 1 pneumocyte with two vacuoles, one of which contains seemingly unaltered organisms. *Arrows* point to basal membrane separating an alveolus (A) from blood vessels (V), one of them containing a leukocyte (L). **(B)** Damage of non-infected endothelium (E) and pneumocyte (P) in the vicinity of an alveolar macrophage (M) containing a phagolysosome (L) with partially degraded organism (*thick arrow*). *Thin arrows* point to blister-like vesicles in damaged cells. Magnifications: **(A)** 5200 ×, **(B)** 7200 ×. (From Khavkin & Tabibzadeh.[11] Reprinted from *Infection and Immunity* with permission from the American Society for Microbiology.)

the most part a mononuclear infiltration, was different from that in an acute bacterial pneumonia.

Evidence for rickettsial involvement in pulmonary lesions in typhus is based on isolation of *R. prowazekii* from the sputum from 4 of 12 patients with epidemic typhus complicated by pneumonia (referred to by Krasnik and Tokarevich[15]), as well as on cases of accidental air-borne laboratory infection. Such aerosol infection was very common in many countries during production of early vaccines since the production of vaccine from infected mouse lungs required intranasal challenge with *R. prowazekii*.[16] The instillation of the rickettsial culture induces in the animal an expiratory reflex with a splash of infected droplets. F. I. Krasnik and K. N. Tokarevich at the Leningrad Pasteur Institute (USSR) told one of us (T. K.) that they contracted air-borne typhus in the late 1940s when evaluating new *R. prowazekii* strains by intranasal challenge of albino mice. An airborne laboratory typhus infection was also reported by Krontovskaya *et al.* in 1946.[17] The disease developed as an acute febrile infection with pneumonia and rash. The immunofluorescent identification by Walker *et al.*[18] of the pathogen in the lung in a fatal case of endemic typhus suggests that *R. typhi* may also be involved in pulmonary lesions in man.

Animal experiments involving the intranasal challenge of albino mice with *R. prowazekii* have demonstrated that this organism indeed resides in cells of the respiratory parenchyma. These cells were originally referred to as epithelium or endothelium (reviewed in Ref. 19). Subsequent ultrastructural studies[19,20] have shown that the main cell type harboring *R. prowazekii* in the mouse lung is the type 1 pneumocyte.

At the appropriate challenging dose, rickettsiae multiply in pneumocytes 3 to 5 days post-inoculation, without inducing an inflammatory response. As in other cells, *R. prowazekii* multiplies directly in the cytoplasm, resulting in reduction of organelles and in eventual destruction of pneumocytes (FIG. 2A). The acute PMN-dominated inflammation associated with circulatory disorders develops upon release of rickettsiae into the alveoli. After elimination of the original pneumonic exudate via the airways, inflammation is transformed into a macrophage and lymphocyte-dominated infiltration with occasional productive thrombovasculitis (FIG. 2B).

In experiments described by Khavkin *et al.*,[19,20] there was no obvious rickettsial multiplication in the vascular endothelium. Occasional membrane-bound rickettsiae are found within endothelial cells (FIG. 3A). This pattern is suggestive of a transcytosis or interendothelial transport of microorganisms into the blood vessels from pneumocytes rather than of residence within endothelial cells. The nondamaged endothelium displayed signs of a vigorous vesicular transport (FIG. 2A). In areas of acute pneumonia, the endothelium was heavily damaged, sometimes destroyed (FIG. 3B). A productive thrombovasculitis observed 5 and 6 days postchallenge does not involve microorganisms.

Chlamydial Infection

Both species of the genus *Chlamydia*, *C. trachomatis* and *C. psittaci*, produce a wide spectrum of lesions in man and animals, including lesions in the respiratory system.[21–23] Furthermore, a new chlamydial isolate, *C. pneumoniae*, strain TWAR,[24] is implicated as an agent of atypical pneumonia in man. As in the case of Q fever and typhus studies, autopsy material has provided little understanding of the pathogenesis of chlamydial infectious processes in lungs and did not define the

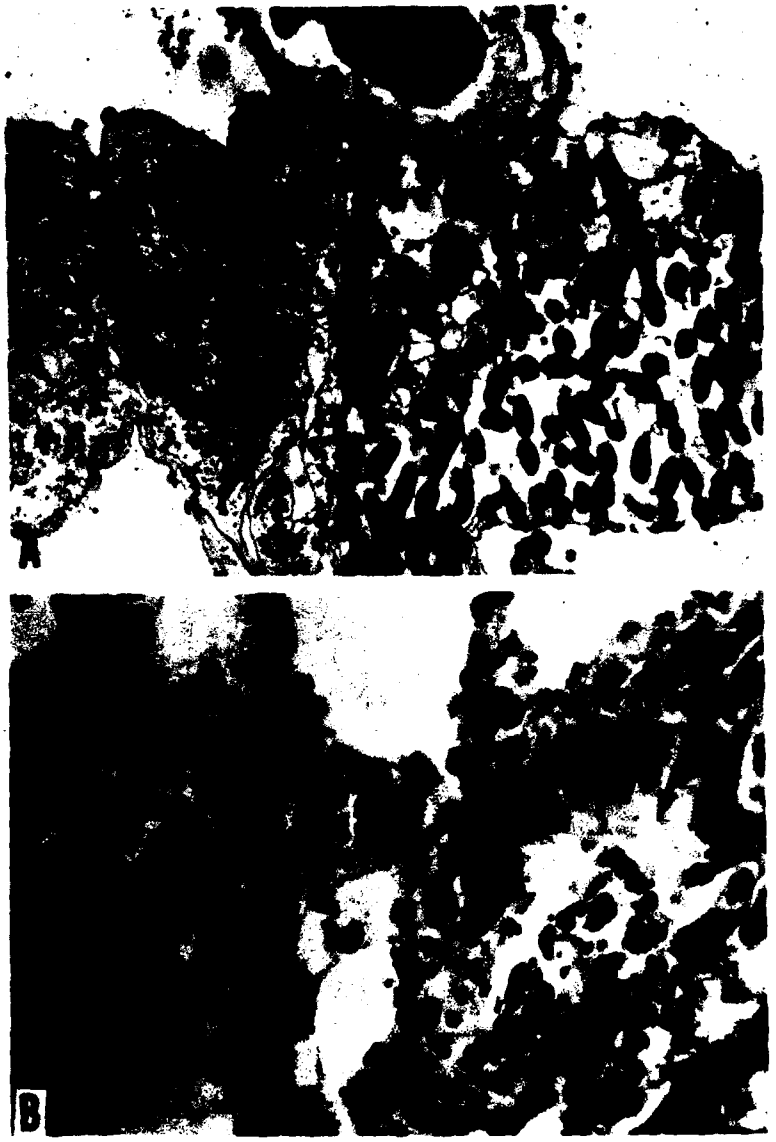

FIGURE 2. *Respiratory parenchyma of mouse lung 72 h (A) and 144 h (B) after intranasal challenge with R. prowazekii, Breinl strain.* **(A)** Detail of the alveolar wall with a type 1 pneumocyte (P) filled with rickettsiae, and adjacent endothelial cell (E) rich in organelles and pinocytic vesicles. *Arrow* points to basal membrane separating these cells. **(B)** *Left arrow* points to a bronchial artery with signs of thrombovasculitis with mostly macrophagic and lymphocyte infiltration of the vascular wall. *Right arrow* points to a bronchus with a mostly leukocytic exudate being eliminated via the airways. Magnifications: **(A)** 7000 ×, **(B)** 780 ×. (Adapted from Khavkin *et al.*[19])

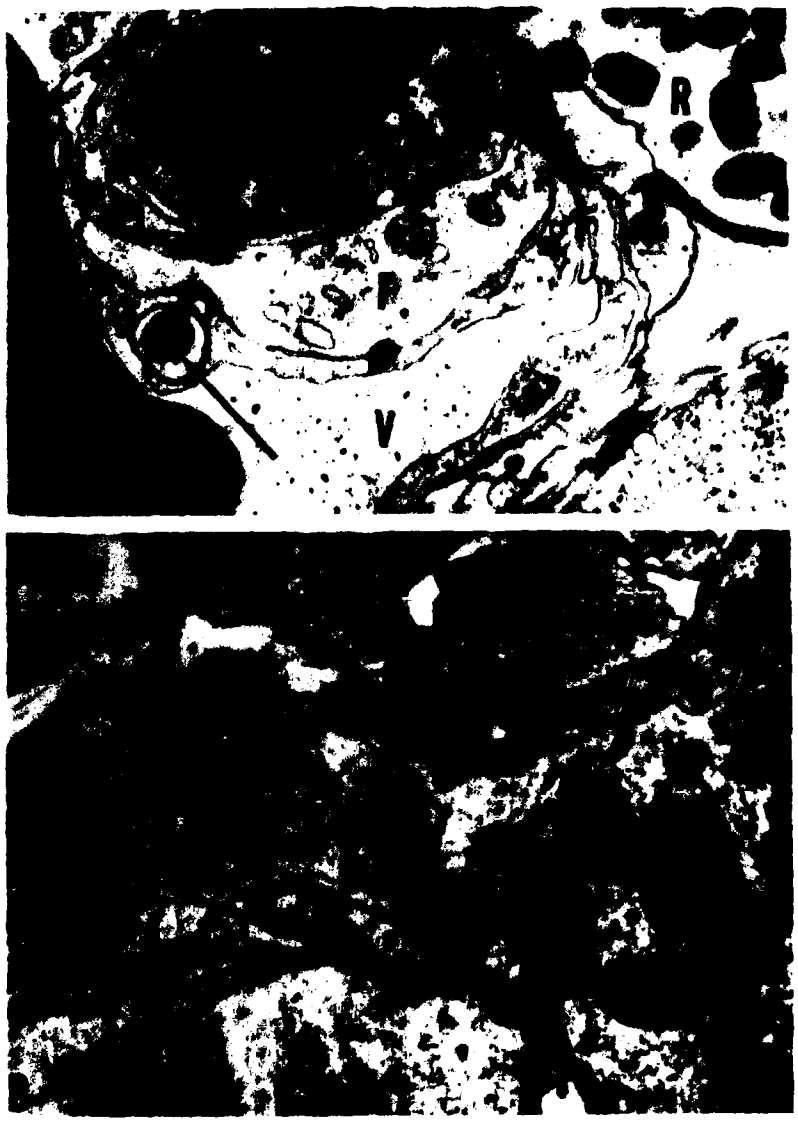

FIGURE 3. Blood vessels in pneumonic foci, 72 h after intranasal challenge with *R. prowazekii*. **(A)** *Arrow* points to a rickettsia bound by a double membrane within an endothelial cell. A septal fibroblast (F) with elastic fibers (E) separates a type 1 pneumocyte, filled with rickettsiae (R), from blood vessel (V). **(B)** Septal capillary with heavily damaged endothelium, and accumulation of thrombocytes. *Arrow* points to a thrombocyte attached to denuded capillary basal membrane. Serous exudate and leukocytes are seen around capillary. Magnifications: **(A)** 12,000 ×, **(B)** 16,000 ×. (Adapted from Khavkin *et al.*[19])

cells that harbor the pathogen. In human psittacosis, a febrile air-borne disease with predominantly respiratory emphasis, the lung is believed to serve as a portal of entry of the agent and is affected secondarily, by hematogenous seeding.[25] Specific inclusions, microcolonies of chlamydial bodies, have been described in unidentified cells attached to the alveolar wall and in cells of the pneumonic exudate.[26] The pathogen has not been demonstrated in lung tissues in the few currently known cases of fatal pneumonia caused by *C. trachomatis* biovars. No information has so far been published concerning host-pathogen interactions in *C. pneumoniae,* TWAR strain, infection.

Some studies in experimental models show, however, that although *C. psittaci* and *C. trachomatis* behave differently in many models,[23] both species are capable of residing within the same cells of the respiratory parenchyma, the pneumocytes. Thus, Tolybekov, *et al.*[27,28] demonstrated that in albino mice, chlamydial colonies appeared in pneumocytes, alveolar macrophages, and bronchial epithelium several days post–intranasal instillation of a culture of *C. psittaci*. The colonies were confined to membrane-bound vacuoles, without obvious damage to the host cell and without an inflammatory response (FIG. 4A). When the infected cells were destroyed, mostly purulent inflammation with subsequent influx of macrophages and lymphocytes developed in their vicinity. Inflammation was accompanied by systemic development of the infectious process.[27–29]

The experimental pulmonary infectious process caused by *C. trachomatis* has largely been studied using the murine pneumonitis biovar. Before the electron microscopic description in 1953 of a complete alveolar epithelial lining,[30] Weiss[31] and Gogolak[32] had demonstrated histologically a close association of agents of murine and feline pneumonitis and meningopneumonitis with the wall of the pulmonary alveolus in mice. A recent ultrastructural study by Coalson *et al.*[33] showed that the cells harboring this pathogen in murine lungs are predominantly type 1 pneumocytes (FIGS. 4B,C) and, in part, bronchial epithelium. Furthermore, Chen and Kuo[34] have identified chlamydial bodies in cells of the pulmonary interstitium. The acute inflammation in lungs developed one or two days post-challenge, upon release of chlamydial bodies from ruptured cells.[32,33] It was gradually changed by lymphocyte and macrophage infiltration, and by the appearance of plasma cells in the respiratory parenchyma. Coalson *et al.*[33] have not observed involvement of blood vessels in the pulmonary infectious process.

INFECTION OF THE PERITONEAL MESOTHELIUM

There are no clinical equivalents to the infection of the peritoneum and its mesothelial cover by the microorganisms discussed in this paper. This is only typical of experimental infection induced via intraperitoneal (i.p.) challenge. The greater omentum represents a convenient model for studying host-pathogen interactions in the peritoneum, because it is involved in peritoneal reactive processes and because it consists of a thin transparent peritoneal fold that can be studied microscopically as a total spread preparation.[35]

C. burnetii *Infection*

Upon i.p. challenge, the local processes in the peritoneum develop quite similarly in both mice and guinea pigs. Khavkin and Amosenkova[36,37] have shown that *C. burnetii* resides largely in resident macrophages and fibroblasts (FIG. 5A).

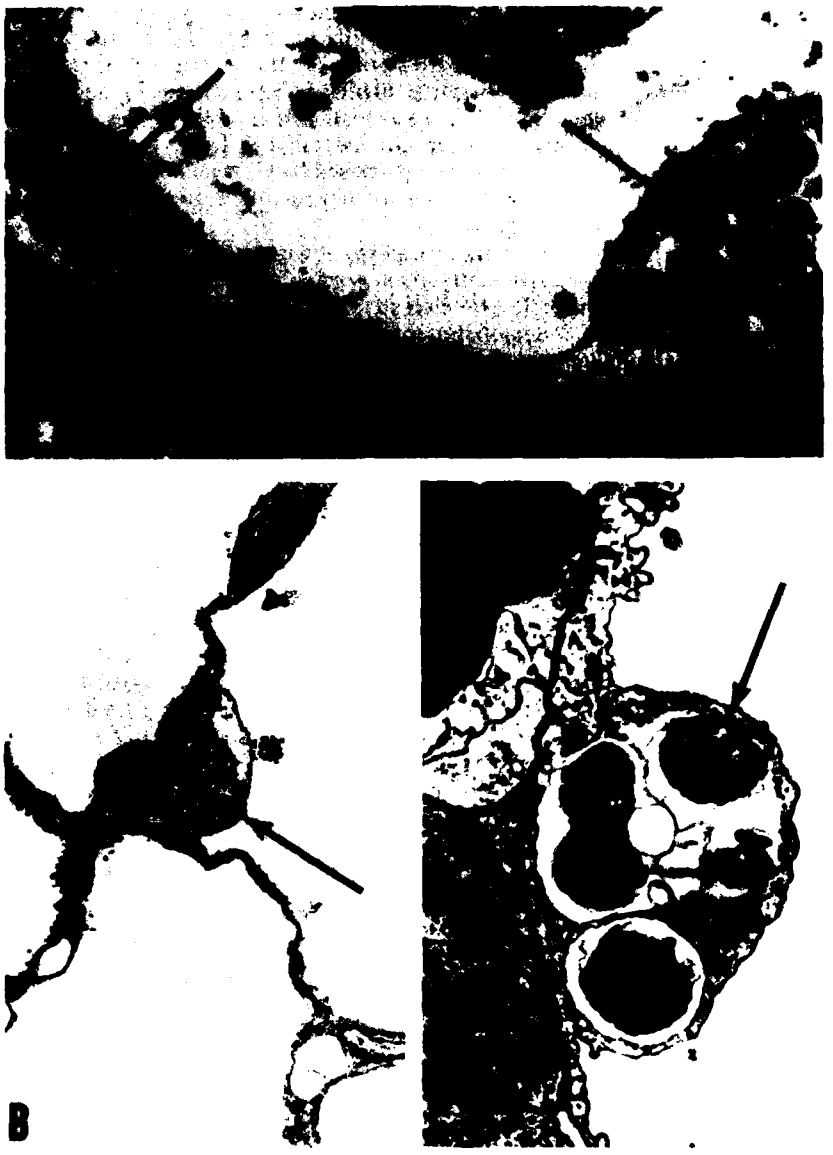

FIGURE 4. Chlamydiae in type 1 pneumocytes in mouse lungs 24 h after intranasal challenge with *C. psittaci* **(A)** or *C. trachomatis* (mouse pneumonia biovar) **(B, C)**. *Arrows* point to pneumocyte areas with chlamydial inclusions that are composed of reticulate bodies, some of them dividing. Infected cells protrude into alveoli that are relatively free of exudate. Inclusions and some individual bodies are confined to membrane-bound vesicles. Magnifications: **(A)** 5000 ×, **(B)** 3400 ×, **(C)** 8500 ×. (Panel **A** is an electron micrograph by A. Tolybekov, reported in Voino-Yasenetski.[29] Reprinted with permission of the publisher. Panels **B** and **C** from Coalson *et al.*[33] Reprinted with permission from the *British Journal of Experimental Pathology*.)

FIGURE 5. *C. burnetii* (Kazachstan strain, phase I) in total omental spread preparations, 8 and 12 days after intraperitoneal challenge. Organisms are identified by staining with basic fuchsin–methylene blue after Gimenez[38] **(A)** and with fluorescent anti-coxiella antibody **(B)**. **(A)** A capillary (C) is apposed by macrophages (M) containing colonies of fuchsinophilic coxiellae (*large arrows*). *Small arrows* point to paranuclear granules, probably mitochondria, that are stained with methylene blue in endothelial cells. **(B)** Specific fluorescence of perinuclear coxiellae colonies (*arrow*) in a mesothelial cell; M, peritoneal macrophages filled with organisms. Magnifications: **(A)** 2000 ×, **(B)** 1200 ×. [From Khavkin & Amosenkova:[36,37] **(A)** Ref. 37, **(B)** Ref. 36. Reprinted with permission of the publisher.]

During the first 5–10 days of infection, coxiellae also appear in mesothelial cells (FIG. 5B). Release of microorganisms from cells is followed by the influx of PMN and macrophages and, in guinea pigs, also by serofibrinous exudation. After the peritoneum has for the most part been cleared of rickettsiae, the inflammation subsides without scarring. Study of total omental spread preparations demonstrates a lack of endothelial involvement in the infectious process, although in small vessels, the endothelium is closely apposed by heavily infected adventitial macrophages (FIG. 5A).

Infection with Typhus Group Rickettsiae

A severe peritoneal involvement in guinea pigs inoculated with *R. typhi* and, to a lesser extent, in those inoculated with *R. prowazekii* is known as the scrotal phenomenon. The appearance of large rickettsia-laden cells in the scrotal peritoneum is typical of this phenomenon.[39] These so-called Mooser's cells were originally considered to be macrophages or mesothelium. Using film preparations of the peritoneum, and omental spreads, Khavkin and Krasnik[40] have observed the formation of Mooser's cells from peritoneal mesothelium in guinea pigs, cotton rats, and mice after i.p. challenge with *R. prowazekii*, Breinl strain.

In mice, which are resistant to i.p. challenge with *R. prowazekii*, the peritoneum is cleared of microorganisms within 48 h post-inoculation. However, occasional mesothelial cells containing rickettsiae can be found. In mice sublethally irradiated by X-rays, the infection becomes generalized. This is accompanied by a massive local multiplication of rickettsiae, largely in mesothelial cells that become indistinguishable from Mooser's cells (FIGS. 6A,B). In guinea pigs and in cotton rats, a massive rickettsial multiplication in the mesothelium is observed during the first week post-inoculation, along with that in fibroblasts and resident macrophages (FIG. 6C), initially without obvious cell damage (FIG. 6D). This is followed by mass destruction of infected cells and by an inflammatory response resulting in fast clearance of the peritoneum of most rickettsiae. Still, rickettsia-bearing mesothelial cells can be seen in guinea pig and cotton rat mesothelium for two weeks, after the acute inflammation has subsided. Immunofluorescent and histologic studies reveal association of *R. prowazekii* with adventitial cells of omental blood vessels during the acute stage of infection, without obvious involvement of endothelial cells (FIG. 6E).

Chlamydial Infection

Early steps of the infectious process in the peritoneum have been studied by Khavkin and Krasnik (unpublished observations) in albino mice challenged i.p. with a lethal dose of an allantoic culture of *C. psittaci*, strain B (provided by I. Terskikh at the Institute of Virology, Moscow). The pathogen was identified in sections and total omental spread preparations with fluorescent rabbit anti–*C. psittaci* IgG and Giemsa stain.

The inoculation induces a transient influx of PMN, which ingest many of the chlamydiae (FIG. 7A). Three hours post-inoculation, some chlamydiae in macrophages and mesothelial cells appeared to be larger than individual elementary bodies in the original allantoic fluid. Obvious chlamydial multiplication, the appearance of large inclusions in mesothelial cells and fibroblasts, is seen as early as 7 h post-inoculation (FIG. 7B). Numerous large inclusions in mesothelial cells,

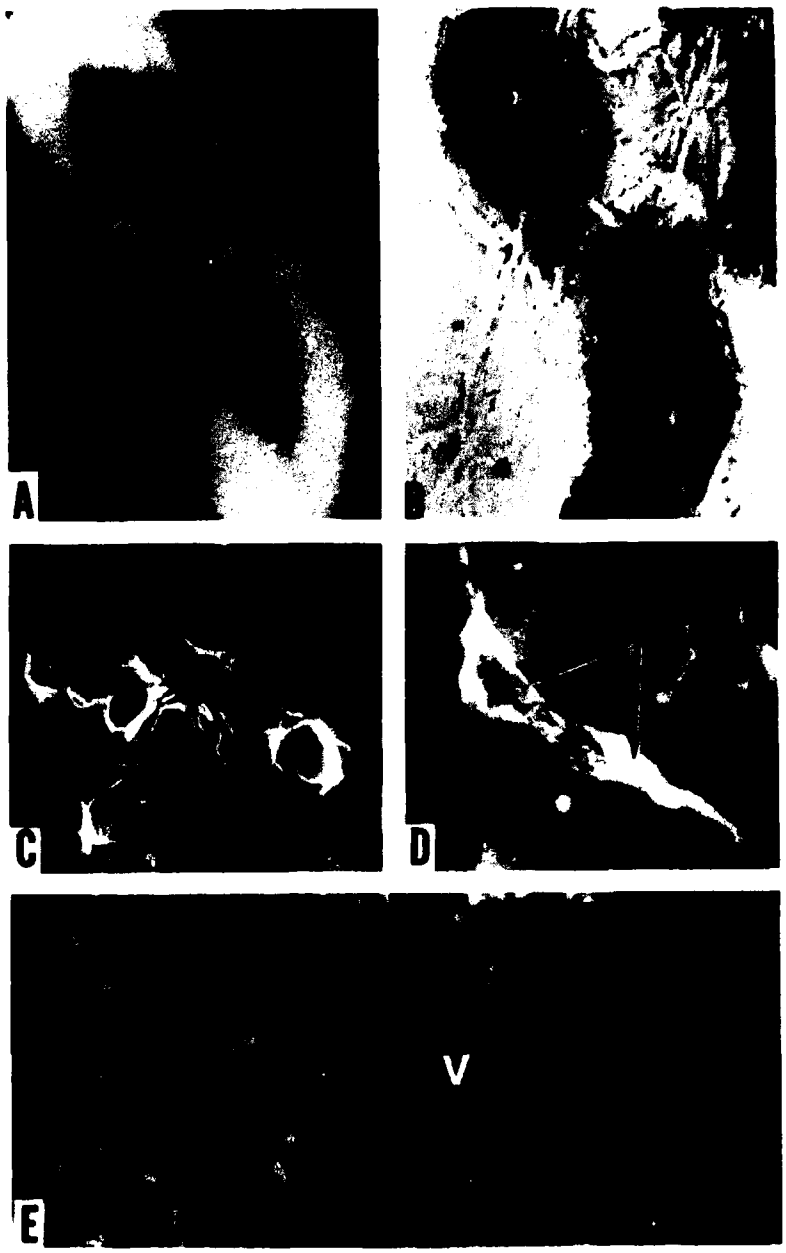

FIGURE 6. Total spread omental preparations from mice **(A, B)** and cotton rat **(C–E)** challenged intraperitoneally with *R. prowazekii*. **(A, B)** The same mesothelial cells filled with organisms, visualized with anti–*R. prowazekii* fluorescent antibody **(A)** or Giemsa stain **(B)**, 10 days after sublethal irradiation given 7 days post-challenge. **(C, D)** Specific fluorescence of rickettsiae in mesothelial cells 72 h after challenge. *Arrows* point to a mitotically dividing cell filled with rickettsiae. **(E)** Overview of omentum, with a blood vessel (V) showing specific fluorescence of rickettsia-laden cells. Endothelium is not obviously infected. Magnifications: 997 ×, **(C, D)** 600 ×, **(E)** 80 ×. (Adapted from Khavkin & Krasnik.[40])

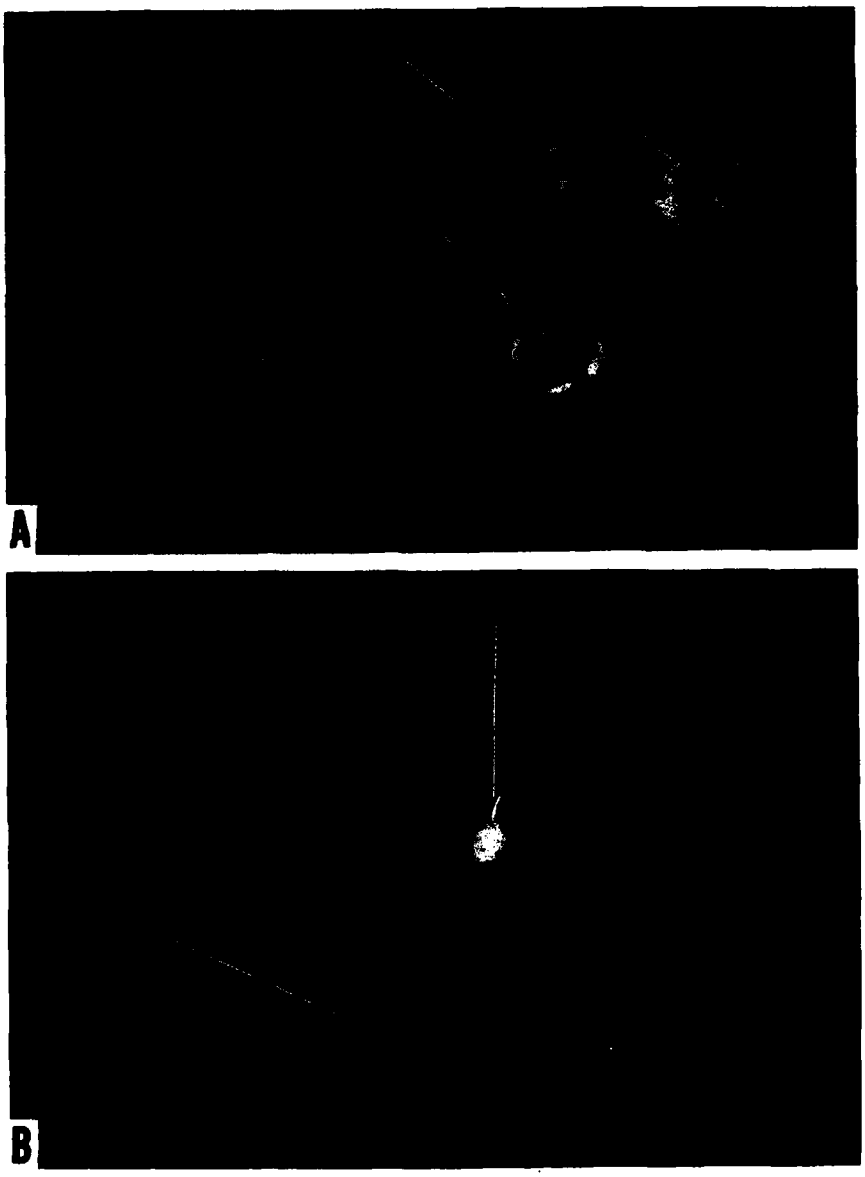

FIGURE 7. Specific fluorescence of *C. psittaci* in total omental spread preparations of mice 3 **(A)** and 11 **(B)** h after intraperitoneal challenge. **(A)** *Arrows* point to PMN filled with ingested bodies. Some extracellular bodies are attached to erythrocytes and to the omental surface. **(B)** Large inclusions (*arrows*) in a fibroblast and a mesothelial cell. PMN contain ingested bodies. Magnification: 200 ×.

fibroblasts, and, to a lesser extent, in macrophages, are found 17 and 24 h post-inoculation (FIGS. 8A, B). Subsequent mass destruction of some infected cells is associated with the start of extensive, mostly purulent inflammation (FIG. 8C). This is followed by the influx of macrophages and lymphocytes, although PMN constitute as many as 30–40% of the exudate cells. Animals die from generalized infection 5–6 days post-challenge. There are no obvious signs of involvement of cells of omental blood vessels in the infectious process in the peritoneum.

DISCUSSION

This review summarizes the observations that the phylogenetically unrelated microorganisms *C. burnetii*, typhus group rickettsiae, and *Chlamydia* spp., share the capability to enter into and parasitize specific cells lining serous membranes and pulmonary alveoli, the mesothelium and pneumocytes, respectively. The affinity of these pathogens for the mesothelium is observed under experimental conditions and has no clinical equivalents in the respective diseases. The affinity for pneumocytes does have clinical implications. Most likely, it underlies the pneumotropism of *C. burnetii*. It is consistent with the presumption by Davydovsky[41] that *R. prowazekii* is a pneumotropic pathogen. And it is suggestive of a pneumotropic feature of both *Chlamydia* species, *C. trachomatis* and *C. psittaci*.

Considering both the clinical and experimental observations, one can speculate that all these pathogens may invade pneumocytes via either air-borne or hematogenous routes. In air-borne diseases such as Q fever and psittacosis, which clinically are sometimes indistinguishable,[8] either route appears to be involved in the development of pulmonary lesions. In epidemic and endemic typhus acquired through the arthropod vector, the lungs are obviously affected via the hematogenous route. Cases of accidental laboratory infection of humans show that airborne typhus is also a strong possibility.

Initially, *Coxiella, Rickettsia,* and *Chlamydia* do not harm the host cell, and they induce neither tissue damage nor inflammatory response until released from the host cell. Such an inapparent beginning, in part, determines the incubation period between microbial invasion into pneumocytes and onset of pathologic and clinical manifestations of pulmonary lesions. At the beginning, acute response to released microorganisms bears little specificity. It differs largely with regard to the intensity of local and systemic toxic effects of the pathogens. Essential differences between infectious processes emerge after immunity mechanisms and the spectrum of pathogenic features of the microorganism are fully engaged. These differences are beyond the scope of the current review.

The inapparent beginning of the infectious processes is not peculiar only to the microorganisms discussed here. It presents an adaptation to the ecological environment that is typical of all the intracellular parasites.[1] A more important problem is whether *Coxiella,* rickettsiae, and *Chlamydia* enter pneumocytes and mesothelium as a result of phagocytosis or whether they are capable of initiating their own entry. The latter mechanism seems to be more likely than the former.

Pneumocytes and mesothelium do not possess phagocytic activity, but they are prone to vesicular transport[42,43] that is associated with the traffic of plasmalemmal vesicles into the cytoplasm. This is a feature of so-called non-professional phagocytes, and it is believed to facilitate entry of microorganisms into these cells.[44] However, *Coxiella* and *Chlamydia* fail to invade vascular endothelium, the third member of the simple squamous epithelia. The endothelium is highly

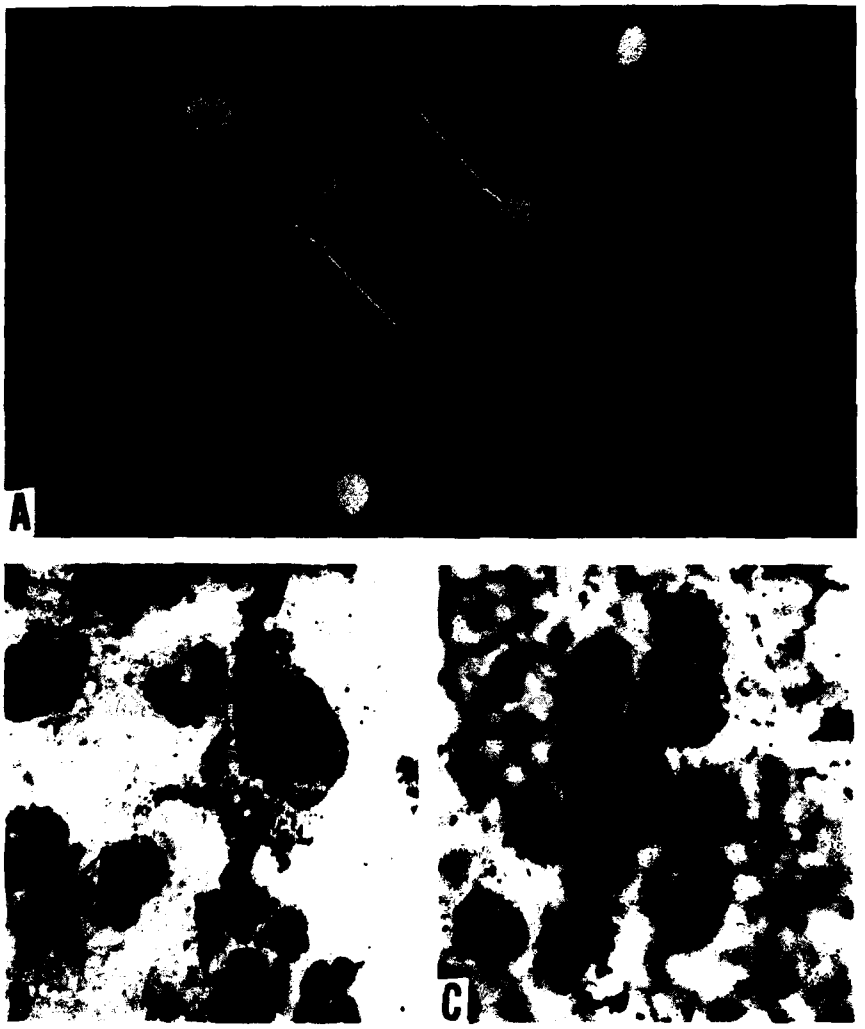

FIGURE 8. Chlamydial inclusions in omental cells 24 h post-challenge as visualized by fluorescent antibody **(A)** and Giemsa stain **(B, C)**. **(A)** Specific fluorescence of inclusions (*arrows*) in mesothelial cells; there is no obvious inflammatory response. **(B)** Infected omental fibroblast; chlamydial inclusion composed of numerous bodies fills entire host cell cytoplasmic space. **(C)** Collection of PMN, some with ingested bodies, around destroyed infected cell. Magnifications: **(A)** 200 ×, **(B, C)** 1400 ×.

prone to vesicular transport, including transcytosis,[45] and is capable of taking up some bacteria.[46] This suggests that vesicular transport plays a minor role, if any at all, in the entry of these microorganisms into pneumocytes and mesothelium. The data presented here also suggest that the vascular endothelium is not a prime target for typhus group rickettsiae, and that rickettsiae are not far from *Coxiella* and *Chlamydia* with regard to their endotheliotropism.

Since the classic work by Wolbach *et al.*,[47] endothelial parasitism by *R. prowazekii* was believed to trigger and sequentially underlie the pathogenesis of destructive thrombovasculitis that is a hallmark of epidemic and endemic typhus.[48] The findings of Wolbach *et al.* were based on studies of Giemsa-stained histologic sections from autopsy cases (FIGS. 9A,B). However, Giemsa and related stains reveal various intracellular granules that are not always distinguishable from microorganisms and may be a cause of unjustified conclusions. Thus, in the early 1920s, Rosenberg[49] and Barykin[50] in Russia identified as rickettsiae granules that were observed in Giemsa-stained cells of human brain, liver, spleen, and endothelium in autopsy cases of epidemic typhus (FIG. 9C). Based on studies of Giemsa-stained sections, Nicolau *et al.*[51] in Romania implicated rickettsiae in an etiologic role in coronary disease. Pinkerton and Maxcy[52] described numerous granules considered to be rickettsiae in Giemsa-stained sections of brain vessels in an autopsy case of sporadic endemic typhus. However, these observations have not been confirmed using more selective staining methods and sufficient optical resolution. Meanwhile, as FIGURE 5A shows, even staining by the Gimenez method sometimes allows the observer to discern both *C. burnetii* and paranuclear granules in vascular endothelium. Contemporary immunofluorescent observations[16,53] demonstrate *R. typhi* associated with the wall of blood vessels but do not precisely identify cells harboring the microorganisms. Current electron microscopic and immunofluorescent data[19,40] show that at the early stage of infec-

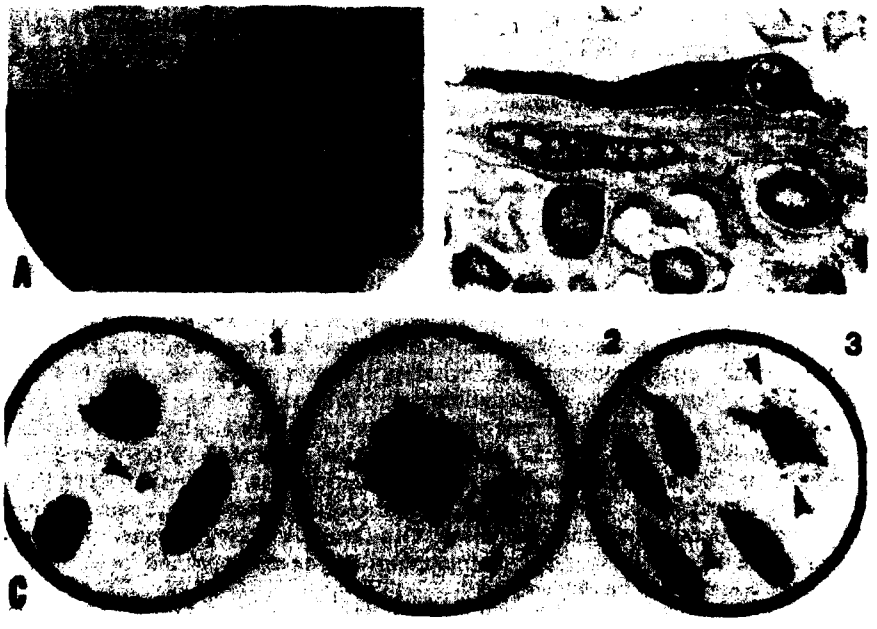

FIGURE 9. Presumed *R. prowazekii* in Giemsa-stained human cells reported by Wolbach *et al.*[47] **(A, B)** and by Barykin and Afanasiev[50] **(C)** in the early 1920s. Photomicrograph **(A)** and a drawing **(B)** were of the same endothelial cell filled with microorganisms (*arrow*). **(C)** Presumed rickettsiae (*arrowheads*) in smears from brain (1), choroid plexus (2), and spleen (3). [**(A, B)** From Ref. 47. Reprinted with permission of the publisher.]

tion in experimental animals, the vascular endothelium may not harbor *R. prowazekii*. This is consistent with the data by Wolbach,[47] who observed vasculitis in infected guinea pigs but was less successful in revealing rickettsiae in the animal's endothelial cells. These data suggest that animal models are not sufficient to study endothelial parasitism in man by *R. prowazekii*, which is a strong anthropophilous pathogen, and that we cannot be sure that what we learn from experimental animal infection applies to human infection. But animal experiments indicate that typical vasculitis may develop independently of endothelial infection by *R. prowazekii*. This speculation is consistent with clinical and autopsy observations during World War II suggesting that vasculitis and rash in epidemic typhus represent immunopathologic events that develop with a delay after onset of infection.[54]

Current data on Rocky Mountain spotted fever (RMSF) suggest that for its agent, *R. rickettsii*, another member of the *Rickettsia* genus, the vascular endothelium is not a prime target cell. *R. rickettsii* does invade the endothelium, but only occasionally and in association with infection of other cells of the blood vessel.[55–57] As Walker[58] has stated, *R. rickettsii* is not particularly well adapted to grow in human endothelium but may affect it accidentally, rather than because of any receptor-mediated tropism. The identification by De Brito *et al.*[57] of the immune complex involving complement in the wall of affected blood vessels in experimental *R. rickettsii* infection suggests that immunopathologic mechanisms are involved in the pathogenesis of vasculitis in RMSF. These authors have compared vascular lesions in experimental RMSF with Arthus-type allergic vasculitis. A similar immune complex–associated vasculitis has also been described in dermal lesions in Mediterranian spotted fever.[59] All these data suggest that endothelial invasion by rickettsiae is rather a secondary phenomenon. Further study of the pathogenesis of vascular lesions in epidemic and endemic typhus should identify the cells harboring rickettsiae, examine for possible immunopathologic phenomena in the vascular wall that might involve rickettsial antigens and complement, and determine the causal relationship of these phenomena.

Both rickettsiae and chlamydiae have been shown in cell culture experiments to be capable of initiating entry into their respective host cells.[1,60] Recent observations suggest that *C. burnetii* is also able to initiate entry into non-professional phagocytic cells.[11] The range of cells in the human and animal body capable of harboring *Coxiella, Rickettsia,* and *Chlamydia* is, however, more restricted than that seen in *in vitro* systems.[61,62] This suggests that *in vivo*, only restricted subsets of cells actually express appropriate receptors. Furthermore, the capability to adhere and enter into a susceptible cell requires genetically encoded determinants of pathogenicity on microorganisms, including adhesive molecules, the adhesins, that interact with receptors on susceptible cells.[2] It appears that invasion into and parasitism within pneumocytes and mesothelium *in vivo* by *Coxiella, Rickettsia,* and *Chlamydia* is also a specific phenomenon that is dependent on respective molecules on these microorganisms and on receptors on susceptible cells, all genetically determined. The nature of these molecules that may appear during convergent adaptation of microorganisms to a common ecological environment, as well as the nature of the respective receptors on pneumocytes and mesothelial cells, well deserves to be determined.

ACKNOWLEDGMENT

The authors are indebted to Diane Young (Interferon Sciences, Inc.) for her excellent secretarial help.

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